Amino Acids (2002) 22: 405–416

Amino
Acids

Effect of Ca^{2+} and Mg^{2+} on the Mn-superoxide dismutase from rat liver and heart mitochondria

V. Pérez-Vázquez[1], **J. Ramírez**[1], **L. Aguilera-Aguirre**[1], **J. C. González-Hernández**[1], **M. Clemente-Guerrero**[1], **S. Manzo-Ávalos**[1], **S. Uribe**[2], and **A. Saavedra-Molina**[1]

[1] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mich., México
[2] Instituto de Fisiología Celular, UNAM, México

Accepted August 16, 2001

Summary. The manganese superoxide dismutase (Mn-SOD) converts superoxide anions to hydrogen peroxide plus oxygen, providing the first line of defense against oxidative stress in mitochondria. Heart mitochondria exhibited higher Mn-SOD activity than liver mitochondria. In mitochondria from both tissues Mn-SOD activity decreased after incubation at low oxygen concentration (hypoxic mitochondria). The effects of free Ca^{2+} ($[Ca^{2+}]_f$) and free Mg^{2+} ($[Mg^{2+}]_f$) on normoxic and hypoxic mitochondria from either organ were tested. In normoxic mitochondria from either tissue, both $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ activated the enzyme, although $[Mg^{2+}]_f$ was less efficient as an activator and the effect was lower in heart than in liver mitochondria. When added simultaneously, high $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ exhibited additive effects which were more pronounced in heart mitochondria and were observed regardless of whether mitochondria had been incubated under normal or low oxygen. The data suggest that $[Ca^{2+}]_f$ plays a role in regulating Mn-SOD in concert with the activation of aerobic metabolism.

Keywords: Amino acids – Liver mitochondria – Heart mitochondria – Mn-SOD – Calcium – Magnesium – Superoxide anion

Introduction

The mitochondrial superoxide dismutase (Mn-SOD) detoxifies superoxide anions ($O_2^{\bullet -}$) by catalysing their conversion to hydrogen peroxide (H_2O_2) plus oxygen (O_2), thus providing the first line of defense against oxygen radicals in mitochondria (Borgsthal et al., 1992). Mitochondria are the main source of $O_2^{\bullet -}$ production and thus Mn-SOD has been considered an essential enzyme in aerobic organisms (Lebovitz et al., 1996).

Homozygous Mn-SOD deletion in transgenic mice results in a lifespan of one to two weeks and in death due to cardiac mitochondrial dysfunction (Lebovitz et al., 1996). In heterozygous mice (Mn-SOD +/−) diminished activity of aconitase, increased oxidation of proteins and DNA and a greater opening probability of the mitochondrial permeability transition pore was reported (Williams et al., 1998).

Within the respiratory chain, $O_2^{\bullet -}$ is produced mainly by the complexes I (NADH-CoQ reductase) and III (Ubiquinol-cytochrome c oxido-reductase) (Turner and Boveris, 1980; Turrens et al., 1985). In these enzymes, the rate of $O_2^{\bullet -}$ production strongly depends on the concentration of auto-oxidizable respiratory transporters and the redox state of the electron transporters (Allen et al., 1997). This becomes very important in the heart, the brain and skeletal muscles which are highly aerobic (Allen et al., 1997).

In mammals, Ca^{2+} controls respiratory chain activity through the regulation of pyruvate dehydrogenase, isocitrate NADH dehydrogenase and α-ketoglutarate dehydrogenase (Hansford, 1985; McCormack and Denton, 1987). The physiological mitochondrial Ca^{2+} concentration in the cell ($[Ca^{2+}]_m$) fluctuates from 0.05 to 2μM. However, during ischemia-reperfusion stress the cytoplasmic free calcium concentrations ($[Ca^{2+}]_f$) rise to exceedingly high levels resulting in $[Ca^{2+}]_m$ as high as 10 to 20μM, damaging cell membranes probably through $O_2^{\bullet -}$ production (Nicholls, 1997).

Oxidative stress additionally results in depletion of Mg^{2+}, which in turn has been reported to be an inhibitor of protein oxidation. Thus, it has been suggested that Mg^{2+} depletion might also play a role in the pathogenesis of oxidative stress (Stadtman and Berlett, 1997). In addition, Mg^{2+} regulates the activity of diverse enzymes or metabolic pathways such as pyruvate-dehydrogenase phosphatase, isocitrate-NADH dehydrogenase, inorganic pyrophosphatase, mitochondrial glutaminase and citrulline synthesis (McCormack et al., 1990; Rodríguez-Zavala et al., 1997).

The relationship between the variations in Ca^{2+} and Mg^{2+} concentration during oxidative stress and the effects of each of these cations on the Mn-SOD activity should be studied as this may constitute a mechanism of cell protection against oxidative stress. In this paper, the effect of different $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ on the Mn-SOD activity was evaluated in mitochondria isolated from either liver or heart. The possible participation of $[Ca^{2+}]_f$ as a physiological regulator of the Mn-SOD is discussed.

Materials and methods

Chemicals

All chemicals were reagent grade and of the highest purity commercially available. Catalase, cytochrome c from horse heart, xanthine oxidase, nagarse, hypoxanthine and superoxide dismutase were obtained from Sigma Chemical Co. (St. Louis, MO. USA).

Mitochondrial preparation

Mitochondria were isolated from either the heart or the liver of male Wistar rats weighing 200 to 250 g. Heart mitochondria were isolated as described before (Moreno-Sánchez and Hansford, 1988). The tissue was digested for 5 minutes using protease type XXVII (Nagarse). The isolation buffer contained: 250 mM sucrose, 10 mM HEPES, and 1 mM EGTA, pH 7.4. Liver mitochondria were isolated according to Hogeboom et al. (1948) with some modifications (Saavedra-Molina and Devlin, 1997). Briefly, the liver was cut into small pieces in a solution containing 220 mM mannitol, 70 mM sucrose, 2 mM MOPS (3-[N-morpholino]propanesulfonic acid) and 1 mM EGTA (pH 7.4). The suspension was homogenized and subjected to differential centrifugation. EGTA was omitted in the two final washes. The last pellet was resuspended and incubated for 10 minutes in the same buffer, except bovine serum albumin grade VI (Sigma) 0.2% was added. After incubating, the suspension was centrifuged at 9,000 rpm in a JA20 rotor (Beckman) for 10 minutes. Protein concentration was determined by the modified method of Biuret (Gornal et al., 1949).

Oxygen consumption

To evaluate the intactness of mitochondria, oxygen consumption in state 3 and state 4 was measured using a YSI model 5,300 oxygraph equipped with a Clark-type electrode at 25°C in a 2.5 ml chamber containing 0.5 mg mitochondrial protein/ml (Estabrook, 1967). For liver mitochondria the reaction mixture was: 100 mM KCl, 5 mM K_2HPO_4, 3 mM $MgCl_2$, 10 mM HEPES, pH 7.4. For heart mitochondria the reaction mixture contained: 120 mM KCl, 20 mM MOPS, 0.5 mM EGTA, 5 mM K_2HPO_4 and 10 mM NaCl, pH 7.4. In both cases, the substrate was 5 mM glutamate-malate and phosphorylation was initiated with 300 μM ADP. Mitochondrial quality was evaluated by ensuring the presence of a high respiratory control (RC). In heart mitochondria RC was 7.4 $\pm$ 2.1 while in liver mitochondria RC was 8.3 $\pm$ 1.6. Thus, mitochondria from both tissues were well coupled.

Incubation of mitochondria under low oxygen (hypoxic mitochondria)

In order to induce effects similar to those observed in hypoxic conditions, mitochondria (1 mg prot/ml) were incubated for 30 minutes at room temperature in a closed container. During this time, the buffer was aerated with a mixture of N_2 (95%) and CO_2 (5%) every 4 to 5 minutes (Arnet et al., 1996).

Sample preparation

Mitochondria (1 mg prot/ml) were incubated for 10 minutes in 50 mM Na^+HPO_4, 100 μM EDTA, 0.2% Triton X-100 to solubilise the enzyme in order to avoid the permeability barriers, pH 7.8. The mixture was centrifuged at 16,000 rpm for 15 minutes in a JA20 rotor (Beckman) and the supernatant was used for Mn-SOD activity determinations.

Mn-SOD activity

Mn-SOD Activity was determined spectrophotometrically following the $O_2^{\bullet -}$ mediated reduction of ferricytochrome c at 550 nm (McCord and Fridovich, 1969). The reaction mixture contained 50 mM Na^+PO_4, 100 μM EDTA, pH 7.8; 10 μM ferricytochrome c, 75 μM hypoxanthine, 150 units of catalase and the amount of xanthine oxidase necessary

to yield an increase in ΔO.D. of 0.025 per min. Total volume was adjusted to 1 ml. One unit of Mn-SOD was defined as the amount of enzyme needed to inhibit the reduction of ferricytochrome c by 50% (McCord and Fridovich, 1969).

Cation concentration

The total concentration of the cations required to obtain the free concentrations reported was calculated by using a computer program that considers the ionic activity of all the components in solution, temperature and pH (WinMax computer program. Version 2.0. Stanford University, CA. U.S.A.).

Statistical processing

Each determination was made by duplicate and averaged. The data are the average ± the standard error of 4 to 8 mitochondrial preparations. All the results were processed statistically and the significance levels were determined by means of a Student's t-test as described under each figure.

Results and discussion

SOD activity in rat heart and liver mitochondria

The SOD activity was measured in the presence of KCN, in order to exclude contamination by the KCN sensitive Cu, Zn-SOD isoenzymes from the extracellular and cytoplasmic spaces. The presence of small amounts of non-mitochondrial SOD in the intermembrane space has been reported (Weisiger and Fridovich, 1973). In our hands, there was only a small fraction of KCN sensitive SOD activity in mitochondria from either tissue (data not shown). Nevertheless, KCN was used in all the subsequent SOD determinations reported here.

The effect of low oxygen concentration on the activity of the Mn-SOD of isolated mitochondria from either liver or heart was determined (Fig. 1). Mitochondria were incubated at room temperature for 30 min under N_2/CO_2. This period of hypoxic condition has been considered to result in reversible inhibition of oxygen consumption while further incubation results in irreversible damage (Tamatani et al., 2000). This effect seems to be mediated by the generation of free radicals (Griffith and Halestrap, 1995), the inhibition of O_2 consumption (Davies et al., 1987) and the synthesis of nitric oxide (Kozniewska et al., 1992). In rat liver mitochondria, incubation for 30 min at low O_2 concentration resulted in decreased Mn-SOD activity to 110 units/mg prot while the control exhibited an activity of 148 units/mg prot. In heart mitochondria, the same period of incubation resulted in a decrease in Mn-SOD to 150 units/mg prot as compared to 228 units/mg prot in the control. Thus, incubation at low oxygen concentration resulted in partial inactivation of the Mn-SOD both in liver and in heart. This would be in agreement with reports stating that low oxygen incubation inactivates other proteins, such effect probably occurs through oxidation of the lateral chains of diverse amino acids (Berlet and Stadtman, 1997). In addition, the generation of peroxynitrite

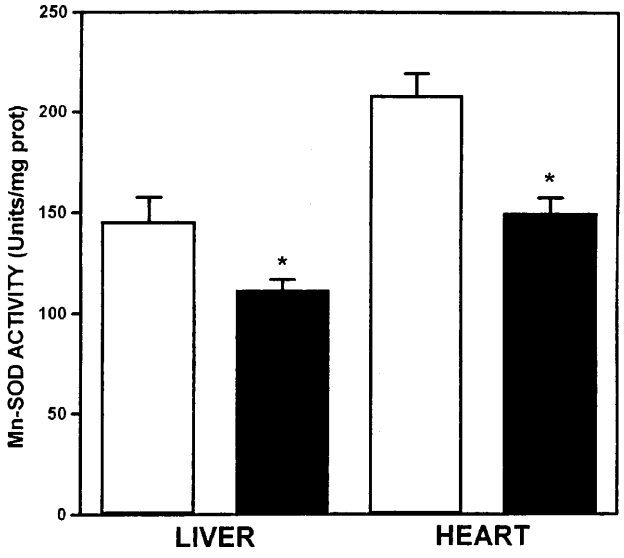

Fig. 1. Mn-SOD activity in rat heart and liver mitochondria. Reaction mixture: 50 mM Na^+PO_4, 100 μM EDTA, pH 7.8; 10 μM ferricytochrome c, 75 μM hypoxanthine, 150 units of catalase. The trace was started by adding the concentration of xanthine oxidase necessary for a ΔO.D. of 0.025 per min. Total volume was adjusted to 1 ml. Mn-SOD activity was determined spectrophotometrically following the $O_2^{\bullet -}$ mediated reduction of ferricytochrome c at 550 nm. Determinations were made in extracts from normoxic mitochondria (empty bars) or hypoxic samples (filled bars). Each bar represents the average $\pm$ SEM of 6 experiments. *($p < 0.05$)

from the reaction between $O_2^{\bullet -}$ and nitric oxide (NO) may contribute to produce further damage (Sharpe and Copper, 1998). In regard to peroxynitrite, it seems to be involved in the nitration of several amino acids. This may be critical for Mn-SOD, as this enzyme is totally inactivated by the nitration of tyr-34. This residue seems to participate in the catalytic cycle of the enzyme facilitating $O_2^{\bullet -}$ access to the active site of the enzyme (Yamakura et al., 1998). Regarding the possible effect of proteases during the isolation procedure or the nitric oxide production by mitochondria, we assayed Mn-SOD activity in mitochondria from both organs incubated in the presence of inhibitors of proteases and of nitric oxide synthesis and obtained no significant differences compared with their respective controls (data not shown).

Effect of $[Ca^{2+}]_f$ on the Mn-SOD activity from rat liver and heart mitochondria

During ischemia, the cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_f$, reaches exceedingly high levels, activating diverse proteases and lipases (Malik et al., 1983). $[Ca^{2+}]_f$ also triggers the permeability transition both in apoptosis and in ischemia probably constituting a major factor in cell death. Thus, it was decided to determine whether variations in $[Ca^{2+}]_f$ from 0.5 to 1.5 μM have effects on the activity of the liver (Fig. 2A) and heart Mn-SOD (Fig. 2B). In

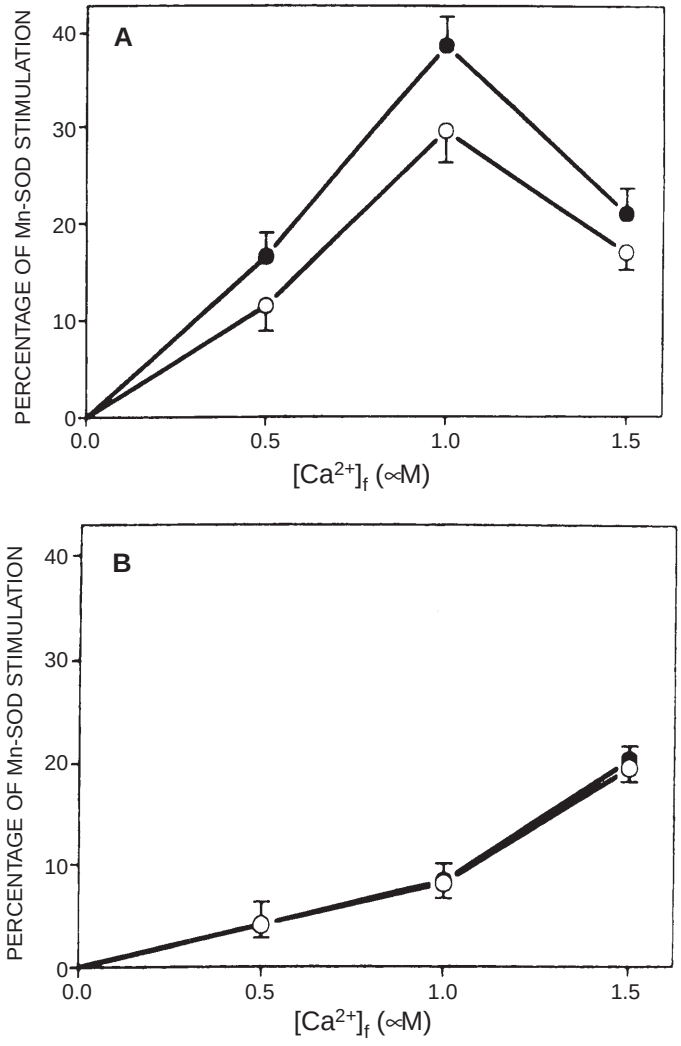

Fig. 2. Effect of $[Ca^{2+}]_f$ on the activity of Mn-SOD in liver and heart mitochondria. Experimental conditions as in Fig. 1. Mn-SOD activity was determined in liver (**A**) and heart (**B**) mitochondria incubated in (●) normoxic or (○) hypoxic conditions. The indicated $[Ca^{2+}]_f$ were calculated as described in methods. Each point represents the average ± SEM of 6 experiments

order to determine if the effects of $[Ca^{2+}]_f$ are different depending on whether mitochondria have been subjected to oxygen limitation, the experiments were performed in normoxic and hypoxic mitochondria.

In normoxic liver mitochondria the Mn-SOD was activated by increasing $[Ca^{2+}]_f$ as follows (Fig. 2A): at 0.5μM $[Ca^{2+}]_f$, Mn-SOD activity increased 17%; at 1μM $[Ca^{2+}]_f$ activation was 39% and at 1.5μM $[Ca^{2+}]_f$ Mn-SOD activation was 20% above control. In hypoxic liver mitochondria, the $[Ca^{2+}]_f$ mediated activation of the Mn-SOD was: at 0.5μM $[Ca^{2+}]_f$ 11% above basal, at 1.0μM $[Ca^{2+}]_f$ Mn-SOD activation was 30% and at 1.5μM $[Ca^{2+}]_f$ activation was 17% (Fig. 2A).

The effects of $[Ca^{2+}]_f$ on the activity of the cardiac Mn-SOD were also evaluated (Fig. 2B). In both hypoxic and normoxic heart mitochondria the effects of $[Ca^{2+}]_f$ were similar. In addition, the $[Ca^{2+}]_f$ mediated percentual activation of the heart Mn-SOD was much smaller than in liver except at the higher $[Ca^{2+}]_f$ tested (1.5μM) where activation was about equal in heart and liver mitochondria.

The decreased Mn-SOD activity observed at 1.5μM $[Ca^{2+}]_f$ in the liver was probably due to the activation of proteases (Malik et al., 1983). In this regard, there are reports indicating that proteins are more sensitive to proteases at high $[Ca^{2+}]_f$ (Malik et al., 1983). The range of $[Ca^{2+}]_f$ in which the greatest Mn-SOD activation was observed is the same which activates the Krebs cycle dehydrogenases (Hansford, 1985; McCormack and Denton, 1987). Thus, it is probable that $[Ca^{2+}]_f$ controls the Mn/SOD in concert with oxidative metabolism as has been recently suggested by Kanoh et al. (2000) in which in bovine tracheal epithelium the generation of superoxide anion is dose-dependent when $[Ca^{2+}]_f$ increases. Also, when neuron mitochondrial $[Ca^{2+}]_f$ was elevated promptly to a micro molar concentrations a subsequent accumulation of reactive oxygen species was detected (Urushitani, et al., 2001). Same results were obtained by Iwama et al. (2001) in human leukemia U937 cells where intracellular acidification, superoxide production and a decrease in the mitochondrial membrane potential were Ca^{2+}-dependent. In our results this would be an efficient method to increase the detoxification of oxygen free-radicals at the time they increase in concentration.

Effect of $[Mg^{2+}]_f$ on the Mn-SOD activity in mitochondria from rat liver and heart

The $[Ca^{2+}]_f$ mediated effects on Mn-SOD (Fig. 2), suggested that the other physiologically abundant divalent cation, Mg^{2+}, might also modulate the activity of Mn-SOD. In this regard, it has been reported that oxidative stress induces Mg^{2+} deficiency in animals and/or cellular cultures, while Mg^{2+} depletion in turn, induces increased protein oxidation (Stadtman and Berlett, 1997). These results suggest that Mg^{2+} has a role in oxidative stress management. The effect of different $[Mg^{2+}]_f$ on Mn-SOD activity was studied in mitochondria from liver (Fig. 3A) and heart (Fig. 3B) which were previously incubated at normal or low oxygen concentrations.

In rat liver mitochondria (Fig. 3A) the effects of $[Mg^{2+}]_f$ from 0.5 to 3.0 mM on the Mn-SOD activity were studied. In normoxic mitochondria, all the $[Mg^{2+}]_f$ tested resulted in a stimulation of 20%, as compared to the control. In low oxygen incubated liver mitochondria, $[Mg^{2+}]_f$ from 0.5 to 1.5 mM activated Mn-SOD by 12% while 3 mM $[Mg^{2+}]_f$ led to 15% activation.

In heart mitochondria (Fig. 3B), Mn-SOD activity increased linearly in the presence of $[Mg^{2+}]_f$ from 0.5 to 1.5 mM to reach an activation of 8% and remain at the same level at 3.0 mM $[Mg^{2+}]_f$. In heart mitochondria, the percentual increase in Mn-SOD activity was the same regardless of whether mitochondria were normoxic or hypoxic.

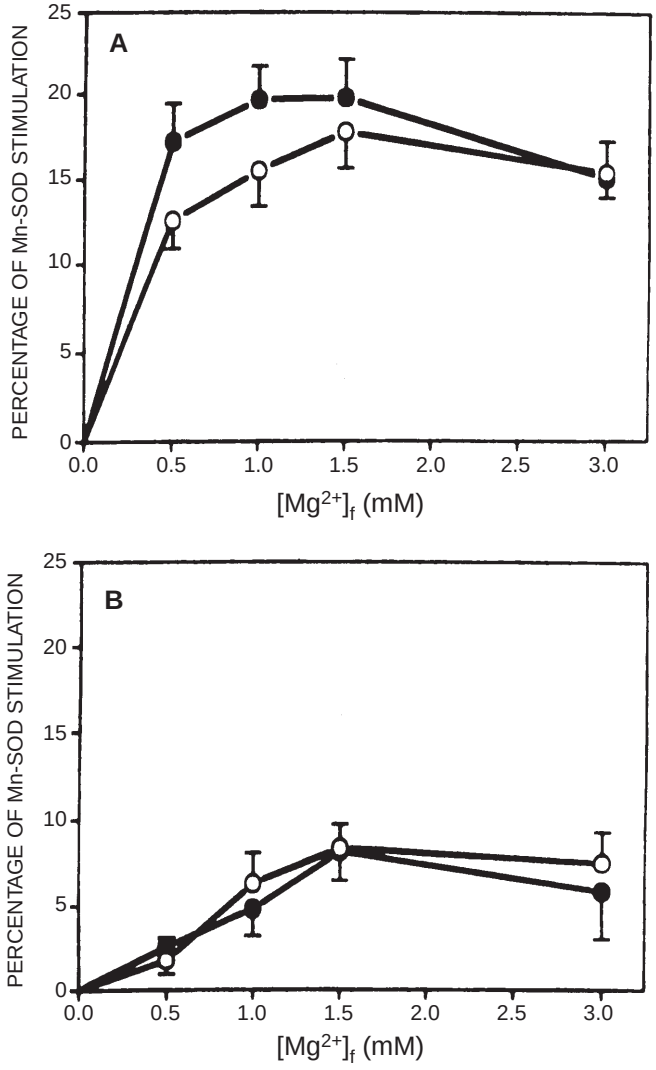

Fig. 3. Effect of $[Mg^{2+}]_f$ on the activity of Mn-SOD in liver and heart mitochondria. Experimental conditions as in Fig. 1. Mn-SOD activity was determined in liver (**A**) and heart (**B**) mitochondria incubated in (●) normoxic or (○) hypoxic conditions. Each $[Mg^{2+}]_f$ was calculated as described in methods. Each point represents the average ± SEM of 6 experiments

Both $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ had a milder effect on the cardiac than on the hepatic Mn-SOD. In addition, incubation at low oxygen did not modify the sensitivity to $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ in the heart Mn-SOD while in the liver enzyme, incubation at low oxygen resulted in less sensitivity to each divalent cation.

The maximal increase in Mn-SOD activity was obtained in the presence of 1.5 mM $[Mg^{2+}]_f$. This same $[Mg^{2+}]_f$ modulates the activity of citrulline synthesis (Rodríguez-Zavala et al., 1997), oxidative phosphorylation (Rodríguez-Zavala and Moreno-Sánchez, 1998) and glutamine sinthetase (Kovacevic

et al., 1995). On the other hand, significant decreases in plasma ionized Mg^{2+} with augment in intracellular organelles were found in patients in chronic stress conditions i.e. increased plasma superoxide anions and malondialdehyde and modified antioxidant defense (Cernak et al., 2000). In our case, the activation exhibited by Mg^{2+} occurs both in normoxia and hypoxia (Fig. 3B).

Effect of the simultaneous increase of $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ on the Mn-SOD activity in mitochondria from rat liver and heart

Interactions between the two divalent cations calcium and magnesium on the cellular level have remained a controversial issue. That this debate continues is due to the incompatibility of certain results obtained in different cell types and under different experimental conditions. For example, calcium-magnesium interactions in the exocrine pancreas, an organ system in which rapid intracellular Ca^{2+} changes occurs, is an example of how these cations can counteract the effects of each other (Mooren et al., 2001). Whereas some authors have reported a potentiation of intracellular calcium signals in the presence of increased Mg^{2+} concentrations (Yoshimura et al., 1996).

In our case, in the Mn-SODs from either liver or heart, the different activation pattern mediated by $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ led us to determine whether the effects of these cations were synergistic (Table 1). When both cations were added at the same time, Mn-SOD activation was 66%. i.e. there was an additive effect of both cations on the enzyme. When the same conditions were analyzed in hypoxic liver mitochondria and the simultaneous addition of both cations resulted in 43% activation. i.e. although milder, additive activation was still observed in hypoxic liver mitochondria.

In heart mitochondria, the concentration of oxygen during incubation did not affect the Mn-SOD activation percentage mediated by $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ or their simultaneous addition. In both normoxic and hypoxic heart mitochondria the combination of both cations resulted in 22–23% activation. Thus, the effects of $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ were additive in both the liver and the heart enzyme, although in the latter the additiveness was much more pronounced.

Table 1. Effect of $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ on the activity of Mn-SOD from rat heart or liver mitochondria. Experimental conditions as in Fig. 1, except the concentration of each cation was calculated for each individual condition as described under methods. Mn-SOD activity is reported in units/mg protein ± standard error. Values are the mean of 6 experiments. Percentual stimulations under each condition are between brackets

Addition	Liver Mn-SOD		Heart Mn-SOD	
	Normal	Hypoxia	Normal	Hypoxia
0	146 ± 9	112 ± 1	209 ± 11	150 ± 12
1 μM Ca^{2+} plus 1 mM Mg^{2+}	242 ± 7 (66%)	160 ± 12 (43%)	257 ± 8 (22%)	185 ± 6 (23%)

The additive effects of $[Ca^{2+}]_f$ and $[Mg^{2+}]_f$ is interesting because in the cell mitochondria $[Mg^{2+}]_f$ is almost constant while $[Ca^{2+}]_f$ varies rapidly.

The Mn-SODs from heart and liver eliminate $O_2^{\bullet -}$. Furthermore, the high aerobic metabolism of the heart requires a higher Mn-SOD activity than the liver (Opie, 1968). In support of this contention, the main goal of our study, Ca^{2+}-Mg^{2+} actions on Mn-SOD activity, adds new information on the subject since we found that Mg^{2+}-induced activity occurs both in normoxia and hypoxia. That both cations showed additive effects despite O_2 absence and that Ca^{2+} seems to modulate the enzyme activity in concert with aerobic metabolism.

The Mn-SOD sensitivity to $[Ca^{2+}]_f$ may be a mechanism by which this enzyme is physiologically controlled in concert with the aerobic metabolism of the cell. Thus, the same $[Ca^{2+}]_f$ that controls the Krebs cycle dehydrogenases (Hansford, 1985; McCormack and Denton, 1987), probably producing more free radicals, would also protect the cell against these free radicals through activation of the Mn-SOD.

Acknowledgements

The authors appreciate the partial financial support grants from CONACYT (29609-N) and CIC-UMSNH (2.5) México.

References

Arnet UA, McMillan A, Dinerman JL, Ballermann B, Lowenstein CJ (1996) Regulation of endothelial nitric-oxide synthase during hypoxia. J Biol Chem 271: 15069–15073

Berlet SB, Stadtman RE (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272: 20313–20316

Borgstahl EO, Parge HE, Tainer AJ (1992) The structure of human mitochondrial manganese superoxide dismutase reveals a novel tetrameric interface of two 4-helix bundles. Cell 71: 107–118

Cernak I, Savic V, Kotur J, Prokic V, Kuljic B, Grbovic D, Veljovic M (2000) Alterations in magnesium and oxidative status during chronic emotional stress. Magnes Res 13: 29–36

Davies KJ, Lin SW, Pacifici ER (1987) Protein damage and degradation by oxygen radicals. IV. Degradation of denatured protein. J Biol Chem 262: 9914–9920

Estabrook RW (1967) Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. Methods Enzymol 10: 41–47

Griffith EJ, Halestrap AP (1995) Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. Biochem J 307: 93–98

Gornal AG, Bardavill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. J Biol Chem 177: 751–766

Hansford GR (1985) Relation between mitochondrial calcium transport and control of energy metabolism. Rev Physiol Biochem Pharmacol 102: 1–72

Hogeboom GH, Schneider WC, Palade GE (1948) Cytochemical studies of mammalian tissues. I. Isolation of intact mitochondria from rat liver: some biochemical properties of mitochondria and submicroscopic particulate material. J Biol Chem 172: 619–635

Iwama K, Nakajo S, Aiuchi T, Nakaya K (2001) Apoptosis induced by arsenic trioxide in leukemia U937 cells is dependent on activation of p38, information of ERK and the Ca2+-dependent production of superoxide. Int J Cancer 92: 518–526

Kanoh S, Kondo M, Tamaoki J, Kobayashi H, Motoyoshi K, Nagai A (2000) Effects of reactive species on intracellular calcium in bovine tracheal epithelium: modulation by nitric oxide. Exp Lung Res 26: 335–348

Kovacevic Z, Day S, Colett V, Brosnan JT, Brosnan ME (1995) Activation of hepatic glutaminase by spermine. Biochem J 305: 837–841

Kozniewska E, Oseka M, Stys TJ (1992) Effects of endothelium-derived nitric oxide on cerebral circulation during normoxia and hypoxia in the rat. Cereb Blood Flow Metab 12: 311–317

Lebovitz RM, Zhang H, Vogel H, Cartwright J, Dionne L, Lu N, Huang S, Matzuk MM (1996) Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci USA 93: 9782–9787

Malik NM, Fenko DM, Iqbal K, Wisniewski MH (1983) Purification and characterization of two forms of Ca^{2+}-activated neutral protease from calf brain. J Biol Chem 258: 8955–8962

McCord MJ, Fridovich I (1969) Superoxide dismutase. An enzymatic function for erithrocuprein (hemocuprein). J Biol Chem 244: 6049–6055

McCormack JG, Denton RM (1987) The role of Ca^{2+} in the regulation of intramitochondrial energy production in heart. Biomed Biochim Acta 46: S487–S492

McCormack JG, Halestrap AP, Denton RM (1990) Role of calcium ions in the regulation of mammalian intramitochondrial metabolism. Physiol Rev 70: 391–425

Mooren FC, Turi S, Günzel D, Schule WR, Domschke W, Singh J, Lerch MM (2001) Calcium-magnesium interactions in pancreatic acinar cells. FASEB J 15: 659–672

Moreno-Sánchez R, Hansford GR (1988) Dependence of cardiac mitochondrial pyruvate dehydrogenase activity on intramitochondrial free Ca^{2+} concentration. Biochem J 256: 403–412

Nicholls DG (1997) Intracellular calcium homeostasis. Br Med Bull 47: 353–358

Opie LH (1968) Metabolism in the heart in health and disease. Am Heart J 76: 681–685

Rodríguez-Zavala JS, Saavedra-Molina A, Moreno-Sánchez R (1997) Effect of intramitochondrial Mg^{2+} on citrulline synthesis in rat liver mitochondria. Biochem Mol Biol Int 41: 179–187

Rodríguez-Zavala JS, Moreno-Sánchez R (1998) Modulation of oxidative phosphorylation by Mg^{2+} in rat heart mitochondria. J Biol Chem 273: 7850–7855

Saavedra-Molina A, Devlin TM (1997) Effect of extra- and intramitochondrial calcium on citrulline synthesis. Amino Acids 12: 293–298

Sharpe MA, Copper CE (1998) Interaction of peroxynitrite with mitochondrial cytochrome oxidase. J Biol Chem 273: 30961–30972

Stadtman RE, Berlett SB (1997) Reactive oxygen-mediated protein oxidation in aging and disease. Chem Res Toxicol 10: 485–494

Tamatani M, Mitsuda N, Matsuzaki H, Okado H, Miyake S, Vitek MP, Yamaguchi A, Tohyama M (2000) A pathway of neuronal apoptosis induced by hypoxia/reoxygenation: roles of nuclear factor-kappaB and Bcl-2. J Neurochem 75: 683–693

Turner JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191: 421–427

Turrens JF, Alexandre A, Lehninger LA (1985) Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. Arch Biochem Biophys 237: 408–414

Urushitani M, Nakamizo T, Inoue R, Sawada H, Kihara T, Honda K, Akaike A, Shimohama S (2001) N-methyl-D-aspartate receptor-mediated mitochondrial Ca(2+) overload in acute excitotoxic motor neuron death: a mechanism distinct from neurotoxicity after Ca(2+) influx. J Neurosci Res 63: 377–387

Weisiger RA, Fridovich I (1973) Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization. J Biol Chem 248: 4793–4796

Williams DM, Remmen VH, Conrad CC, Huang TT, Epstein JC, Richardson A (1998) Increased oxidative damage is correlated to altered mitochondrial function in hetero-

zygous manganese superoxide dismutase knockout mice. J Biol Chem 273: 28510–28515

Yamakura F, Taka H, Fujimura T, Murayama K (1998) Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. J Biol Chem 273: 14085–14089

Yoshimura M, Oshima T, Matsuura H, Ishida T, Kambe M, Kajiyama G (1996) Potentiation of the intracellular Ca^{2+} response to arginine vasopressin by increased cytosolic-free Mg^{2+} in rat vascular smooth muscle cells. Biochim Biophys Acta 1312: 151–157

Authors' address: Alfredo Saavedra-Molina, Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mich. 58030, México, Fax: 52-443-326-5788, E-mail: saavedra@zeus.umich.mx

Received April 2, 2001